

*The Yolk Nucleus in Cymatogaster aggregatus Gibbons.*

*By Jesse W. Hubbard.\**

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The so called yolk nucleus is a compact, irregular mass of granules found in the yolk of the eggs of certain animals. It lies outside the germinal vesicle and usually takes a deep stain similar to the nucleolus within the germinal vesicle.

It has been seen in the eggs of Cnidarians (Häcker and others), Nematodes (Monticelli), Sagitta (Hertwig), Crustaceans (Weismann and others), Myriopods (Ludwig), Insects (Balbiani), Arachnids (Carus, Ludwig and others), Lamellibranchs (Ihering), Gastropods (Balbiani), Teleosteans (Van Bambeke), Batrachians (Cramer, Carus and others), Reptiles (Eimer), Aves (Cramer and others), Mammals (Schäfer). It is probably a normal structure of fish eggs, but on account of unfavorable conditions its complete history has not been traced in any species.

The problem we wish to solve is : (1) How does this body originate, (2) what becomes of it, (3) what function has it in the egg? There are many difficulties to the solution of the problem by ordinary fish eggs. Their great size, complexity of structure owing to accumulation of yolk, close proximity to each other and liability to crumble in sectioning—all are a detriment to its study.

Cymatogaster has in these respects many advantages over all others. (1) The eggs of this genus are the smallest of fish eggs, hence a few sections show the entire egg. The eggs of Cymatogaster reach a maximum size of .3 mm. in diameter; while other fish eggs average about 1 mm. in diameter. (2) There are comparatively few eggs in each ovary, so they are not distorted by crowding. (3) In their natural state, if uninjured by reagents, they are perfectly spherical and thus admit of easy measurement. (4) There is but little yolk when ripe and hence they may be sectioned without crumbling. (5) The nucleus seldom contains many nucleoli. It generally has but one distinct nucleolar spot. In very young eggs, and those about ripe, a few others may be seen, not more than four or five. (6) The last and most important advantage these eggs possess is the fact that the yolk nucleus is conspicuous from the time the eggs have reached a diameter of 20  $\mu$  until maturation and even beyond that to the closing of the blastopore!

The observations were made on ovaries collected by Dr. Carl Eigenmann on the coast of California. They represent individuals from 17 mm. to 140 mm. in length and were preserved chiefly in June, July and August and in October, November, December, January and February. Most of them were preserved in Flemming's strong mixture of osmic-

\* Contributions from the Zoölogical Laboratory of the Indiana University, under the direction of Carl Eigenmann, iii.

chromic-acetic. A few others in platinum chloride and corrosive sublimate. Those killed in the two last mentioned were not very favorable for the study of the yolk nucleus. Some of the ovaries were stained in toto by hæmatoxylin. Others were stained on the slide by eosin. This is an excellent stain for eggs near maturity. The former was the better for younger eggs. After clearing with clove oil and infiltration in paraffin they were cut and mounted in Canada Balsam.

I made a number of experiments with different staining media. Neither mythyl green, mythyl violet, dahlia or safrinin sufficiently differentiated the tissues and the body I wished to trace. I found, however, that mythyl violet did not stain anything in the ovary, but did stain the outside muscular tissue and the spermatazoans contained in the ovary, the latter being stained a bright violet. Using alcoholic eosin and methyl violet as a double stain rendered all the tissues red except the spermatozoöns, which were a bright violet.

I have examined the ovaries of very small fish measuring 17 mm., 29 mm., 35 mm. and 40 mm. in length. In none of the largest eggs of these did I find any trace of a yolk nucleus. In a fish 45 mm. long I found a few eggs which seemed to have this body, but not at all distinct, as the ovary was not well preserved. The adult fish reaches a length 140 mm. In a fish 70 mm. long I found this body quite distinct. So it must appear in the egg when the fish is between 40 mm. and 70 mm. in length. But, as I had no specimens between these two sizes, I cannot definitely determine just when it appears in the egg.

In the young, 40 mm. and less in length, the largest eggs measure  $35\mu$ ,  $30\mu$ ,  $25\mu$ . In none of these eggs was the body visible in the protoplasm of the egg. But eggs about the same size as above found in an ovary of an adult fish showed clearly the presence of this body. This early stage was not seen in all the ovaries sectioned. The one in which it was seen was taken October 21, killed in osmic-chromic acetic, stained in hæmatoxylin, cleared with clove oil and mounted in Canada Balsam. The smallest egg in which the yolk nucleus was observed was  $20\mu$  in diameter, *i. e.*, it was smaller than the largest egg in the ovary of the small fish 40 mm. in length, in which the yolk nucleus had not appeared. In these eggs of the adult fish the protoplasm around one side of the nucleus takes a deep stain. It appears as a crescent-shaped body, fitting very closely to one side of the nucleus and forming a kind of cap (Fig. 1, yk. nl.). Another egg of the same measurement shows a little more advanced stage. The body is more definite in shape, not of so pronounced a crescent form and appears to have enlarged considerably (Fig. 2, yk. nl.). The next important change is seen in a slightly larger egg,  $25\mu$  in diameter (Fig. 3, yk. nl.). Here the body has assumed an oval form without any definite cell wall or hard outline. Although usually touching the nucleus it seems to have no further connection with it. In this egg it is quite large, measuring  $19\mu$  in minor axis and  $12\frac{1}{2}\mu$  in major axis. From this time the body seems to have severed its connection with

the nucleus, and during the remainder of the egg's immaturity gradually moves away from the nucleus. From this fact and its early relation to the nucleus it seems evident that it must have originated from the nucleus. But there is another more potent proof that this is its origin. The largest eggs in the young fishes 17 mm., 29 mm., 35 mm. and 40 mm. in length measured as follows :

Diameter of egg .....	35 $\mu$ .	Diameter of nucleus .....	20 $\mu$ .
" " .....	30 $\mu$ .	" " .....	17 $\frac{1}{2}$ $\mu$ .
" " .....	25 $\mu$ .	" " .....	15 $\mu$ .

In other words, the diameter of the nucleus is more than half the diameter of the egg. It will be remembered that none of these eggs contained a yolk nucleus. The smallest eggs in the adult fish containing a yolk nucleus measured as follows :

Diameter of egg .....	20 $\mu$ .	Diameter of nucleus .....	10 $\mu$ .
" " .....	25 $\mu$ .	" " .....	12 $\frac{1}{2}$ $\mu$ .
" " .....	30 $\mu$ .	" " .....	15 $\mu$ .

That is, the nucleus has now been reduced to a diameter equal to one-half that of the egg. If this body originates from the nucleus as an extrusion of a part of its substance we should expect the nucleus to be relatively larger to the size of the egg before it appears than after its appearance. This is exactly the case in these eggs (compare nucleus in Fig. 1a and Fig. 3). It will be seen from the two sets of measurements given above that in the eggs in which this body is not visible the diameter of the nucleus is always *more than half* that of the egg, while in the eggs in which it is visible the diameter is *just half* that of the egg. This relation is true in all the measurements I made.

Take an egg with a diameter of 25  $\mu$  as a typical size of the smallest eggs in the adult fish and the largest in the small fish. To simplify operations I have taken the number of divisions of the mikrometer instead of the absolute measurement of the egg. Then the sizes of the eggs are expressed as follows :

Diam. of egg in adult fish .....	(s) 5.	Diam. of nucleus .....	2 $\frac{1}{2}$ .
" " small " .....	(o) 5.	" " .....	3.

The dark body (*e*) in the protoplasm of the egg being an oblate spheroid measures 2 in conjugate axis and 2.375 in tranverse axis.

Now the solid contents of the nucleus in *o* ought to equal the solid contents of the nucleus *s*, plus the solid contents of the dark body *e*, or  $o = s + e$ .

Formula for contents of a sphere is  $\nabla = \frac{1}{6} \pi D^3$ .

" " " " oblate spheroid is  $\nabla = \frac{4}{3} \pi a^2 b$ .

$\nabla$  = volume.

D = diameter.

*a* = semi-transverse axis.

*b* = semi-conjugate axis.

Substituting values in the formula for the sphere  $\nabla$  of the nucleus of egg  $o = 14.1372$ .  $\nabla$  of the nucleus of egg  $s = 8.18125$ .  $\nabla$  of the dark body  $e$  (or the oblate spheroid)  $= 5.90704 +$ . Therefore  $o$  ( $14.1372$ )  $= s$  ( $8.1825$ )  $+ e$  ( $5.9070 +$ ), leaving an error of only .049, which amounts to very little when considering that each division of the mikrometer only equals .005 mm. and that the outlines of the dark body not being very definite it was difficult to get the precise measurement.

I think this clearly proves that the body originates from the nucleus. But I do not believe it is by ordinary cell division with the formation of a karyokinetic figure. It seems to be a general extrusion of substance from the nucleus.

It will be seen from an inspection of Figs. 4-12 that this body is considerably smaller in these stages than in smaller eggs (Fig. 3). Whether this is due to reagents causing a shrinkage or whether due to a condensation of substance I am unable to determine. After the egg is  $40 \mu$  in size (Fig. 5), at which time the body has reached its minimum size, it continues to constantly increase through all of the other sizes. It is nearly always irregular in shape and very seldom has a hard outline which might represent a cell wall.

Sometimes it assumes very peculiar shapes (see Figs. 7, 9, 10, 13). The most aberrant form is represented in Fig. 9, where it seems to have a long filament or tail, which is only seen by careful focusing.

Cymatogaster is viviparous. The eggs of this fish ripen during the months of December, January and February. In an ovary taken on October 21 they range in size from .02 mm. to .2 mm. The eggs of November 2 begin to show signs of ripening (Fig. 17 ( $\alpha$ )). Here the nucleus is a little eccentric. The zona radiata is quite distinct and the follicle is well formed. At this stage of maturity there is a peculiar circular region shaded much more darkly than the remainder of the vitellus. This always appears on the side of the nucleus next to the yolk nucleus and opposite the point of the egg to which the nucleus seems to be moving. The yolk nucleus under consideration is quite close to the periphery of the egg.

November 30 some of the eggs become about ripe. Fig. 18 represents one which will probably be ripe in January. In this egg the follicle is much developed, the nucleus has become very eccentric and the yolk nucleus is nearly touching the periphery of the egg. Up to this time the constant tendency of this body has been to become further and further removed from the nucleus towards the periphery of the egg.

In the stages indicated by Figs. 19 and 20\* the body has become slightly changed in appearance. These eggs are nearly ripe. The nucleus has reached its limit of eccentricity, and in one stage (Fig. 20) has lost its cell wall. I have merely indicated its position, which is seen in another section. The yolk is beginning to collect at the entodermic pole of the egg, *i. e.*, around the yolk nucleus.

\* These and succeeding figures are based on preparations made by Dr. Eigenmann.

In the ripe egg (Fig. 21) the body in question is found surrounded by the yolk. This egg was in the act of giving off the second polar globule. The latter is not figured because seen in another section.

After the egg is matured this body assumes many curious and interesting shapes (Figs. 21-26). In Fig. 21 the yolk nucleus seems to have broken into large granules, several of them being grouped into a dark mass (yk. nl.). The whole region including the granules is somewhat shaded.

After the egg has segmented into two cells, the appearance is nearly the same as that just described, except the granules are not quite so large and prominent (Fig. 22). To this time the yolk nucleus has shown a granular structure, but as I have represented in the remaining figures it loses that structure and appears to be homogeneous.

In the stage represented in Fig. 23 this yolk nucleus has entirely changed its appearance. I did not have the sections to show the intermediate stages. At this time the egg has segmented into 32-64 cells. The yolk nucleus and its vicinity shows four different degrees of shading. The centre of what seems to be the yolk nucleus proper is of a lighter shade than the margin which is quite dark. Outside of this there is a still lighter shade than that within the body. Beyond the last is another still less distinct.

When the egg has segmented into many cells, perhaps 600 (Fig. 24), the yolk nucleus is larger and shows only one distinct shading.

Fig. 25 shows the greatest peculiarity of any. Here the blastopore is nearly closed. The body in question is somewhat pear-shaped and has a few small vacuoles scattered through it. It does not have such a distinctly granular structure, but is of a uniformly dark shade. It seems to form a kind of plug to the blastopore. The lower edge of this body is marked by a distinct outline, but the upper edge does not have such an outline. For a considerable region in its vicinity there are different degrees of shading. Also near the upper side there is a heavy shading which seems to consist of minute granules.

After the closing of the blastopore (Fig. 25) the yolk nucleus is much reduced in size, the shadings have disappeared and there is seen only a number of granules. The yolk also contains several similar granules scattered through it. Here it seems evident the yolk nucleus has reached the final stage of disintegration. It does not appear in any of the later stages of development. I think that the different appearances this body presents after segmentation of the egg begins are due to the fact that it is in the process of disintegration. The different depths of shading probably indicate the scattering of its substance through the yolk.

This body seems always to be situated at or nearer the entodermic end of the pole of the egg. Its position is constant during all its stages of growth. It may be utilized in determining whether the axis of the egg has any definite and uniform relation to the axis of the ovary. A compari-

son of the axis of eggs as determined by the position of the yolk nucleus shows that the axis of the egg has no uniform relation to the axis of the ovary.

#### SUMMARY.

1. This body originates from the nucleus in very small eggs in the adult ; soon after the cell becomes fully differentiated as an egg.

2. It constantly moves from the nucleus towards the entodermic pole of the egg which it reaches when the egg is ripe.

3. It is situated at the entodermic pole of the egg at maturity and during later stages.

4. It is capable of growth condensing apparently when very young and then continuing to grow to considerable size.

5. It is of definite chemical composition, having great affinity for certain stains.

6. It remains in the egg until the closing of the blastopore and then breaks up and disappears in the yolk.

7. It is found in the eggs of many animals and has been figured as belonging to the spermatogonium or male cell. See especially *Zeit. für Wissen. Zool.*, Vol. li, Taf. xxxvi ; also *Arkiv. für Mikro. Anat.*, Vol. xxxix, Taf. xxi.

The majority of papers on embryology which mention this body dismiss it without much comment. However, there are a few writers who attempt to explain its function in other animals. The consensus of opinion seems to be that it is the centre of yolk formation. But there is no direct evidence that such is the case. It is mere speculation from the fact that it is found in the protoplasm of the egg, before the yolk is formed. In the eggs of *Cymatogaster* I have never seen any evidence that it gives rise to the yolk. The yolk globules are scattered through the protoplasm, seeming to appear equally in all parts of the egg. As the yolk gradually forms it is homogeneously distributed in different sectors of the egg until maturation. Then the yolk collects at the entodermic pole of the egg, where the yolk nucleus has become located long before.

The close association which this body has with the yolk would seem to indicate that it is in some way connected with it. But how? I think in many of the eggs in which this body has been seen it is not at all functional. But from the length of time it remains in the eggs of *Cymatogaster* and from the wonderful changes it undergoes in growth it would seem to be functional in this egg. Dr. Eigenmann has shown that these eggs mature very rapidly and hence have a small amount of yolk. This fact may account for the yolk nucleus remaining so long after segmentation begins. If the egg matured very slowly and allowed the formation of a large amount of yolk this body would probably disappear before the egg is ripe, as it does in other fishes.

Another interesting question arises as to what determines its position

exactly at the entodermic pole of the egg. It seems to tend to that definite position when it first leaves the nucleus. But without any question it takes up its station there before the nucleus begins to move towards the periphery of the egg, as the nucleus always moves in a directly opposite direction from the body. In all eggs the yolk collects at the entodermic pole of the egg. Does this peculiar body serve as an attraction for the yolk in this egg? Or is it a mere coincidence that it is in the midst of the yolk and has no particular connection with it?

In conclusion, I would say that I cannot definitely determine its function. I think the body is homologous with the meganucleus in protozoans. It is the vegetative portion of the egg given off from the nucleus when the egg cell has become fully differentiated as such. That which remains of the nucleus is homologous with the micronucleus in protozoans. It is the animal part of the egg which is further concerned wholly with the reproduction of the species.

#### HISTORICAL REVIEW OF THE LITERATURE ON THE YOLK NUCLEUS.

Hoffman ('78, p. 545) in his studies on the young ovarian eggs of amphibians saw the yolk nucleus in *Rana esculentia*. He merely mentions it and describes it as a round, dark, granular body within the yolk. He says it is not seen in all amphibians.

Balbani ('79) has had some very peculiar views on the origin of this body. His ideas being now so untenable, I quote this more as a curiosity than as shedding any light on the true origin. He considers the follicular cells as homologous to the spermatoblasts. The yolk nucleus corresponds to the spermatoc elements. One, becoming free from the follicle, penetrates the yolk. When it first enters it leaves a sort of canal behind it, which is soon closed up by the surrounding yolk. It is a sort of spermatozoid and partially fertilizes the egg preliminary to the true fertilization which takes place later. In parthenogenetic eggs this body would perform the function of the male element. If his descriptions are based on facts the body he described is not homologous with the yolk nucleus of authors in general.

Schäfer ('80) describes and figures this body in the eggs of the rabbit. He thinks he saw some connection between the nucleus and the yolk nucleus. He believes that the latter is derived from the former, but not by a process of ordinary cell division.

Schütz ('82) has given an extended review of this body as seen in various classes of animals. But he seems to have no adequate conception either of its origin, function or fate. He remarks that the yolk may have produced it and that it later serves as nutrition for the yolk. The observations were made on isolated specimens of fresh eggs. No sections were made nor was the body traced out in any single species. He has, however, carefully compiled a large list of animals in which it has been seen, and has described its structure and appearance in many of them. His refer-

ences to the literature on the subject is extensive, but, much of it being inaccessible to me, I have made no note of what I could not personally examine.

Van Bambeke ('83) studied this body in the eggs of *Luciscus rutilus* and *Rhodeus amarus*. He says very little as to its origin, but thinks it may come from the nucleus. His figures show it to be connected with the nucleus by a funnel-shaped tube, the large end of which encloses the nucleus and the small end touches the yolk nucleus. From this appearance it would seem to be connected in some way with the nucleus. He seems to think this body is the centre of the yolk elements.

Balbani ('83, p. 676) found this body in the eggs of *Geophiles*. In these eggs the nucleus sends out prolongations. These break off close to the nucleus and in turn they break up into large granules. These take a deep stain. These granules, he thought afterwards, left the vitellus and became the cells of the follicle. In this theory he was supported by Fol, Roule and Sabatier. There was one granule, however, that became differentiated from the others, and instead of forming a cell of the follicle remained in the vitellus and became what is known as the yolk nucleus. He thought its function was to originate the yolk. He says: "The first granulations of yolk are produced on the surface of this body and afterwards distribute themselves to all parts of the egg." In some animals he finds lines radiating in all directions from this body. Forming the limit to these radiating lines are concentric layers of substance.

Balfour ('85, pp. 21, 50) describes the yolk nucleus as seen in *Araneina* by Ludwig and others. He says it always disappears before development commences. He remarks that it is probably connected in some way with the nutrition of the ovum. This body is not found in all genera of *Araneina*.

Haeker ('92, p. 251) having studied this body in *Aequorea forskalea* calls it the metanucleolus. In the ovarian eggs it was present within the nucleus as the nucleolus. One half hour after being laid the nucleus appeared to extrude this body. He traced the body in the blastomeres until a late stage in cleavage. The author thinks homologous structures have been seen in other Medusæ. He considers the paracopulation cells of Daphnids described by Weismann and Ishikawa as homologous with the metanucleus of Medusæ. Haeker did not observe any division or any radial arrangement of protoplasm around this body.

Monticelli ('92) has studied this structure in the ova of *Distomum veliporum* and *D. richiardi*. He concludes that the vitelline nucleus is a cytoplasmic product, a nutritive differentiation probably acting as a centre in the formation of yolk.

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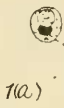
Plate I.

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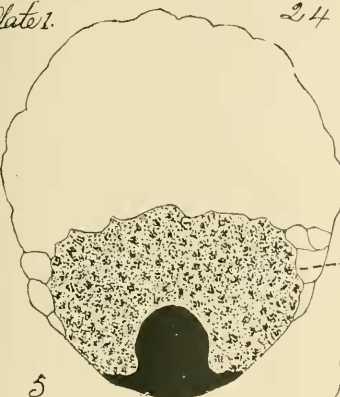
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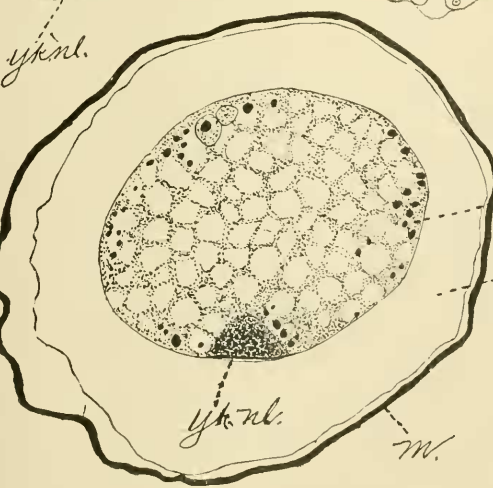
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